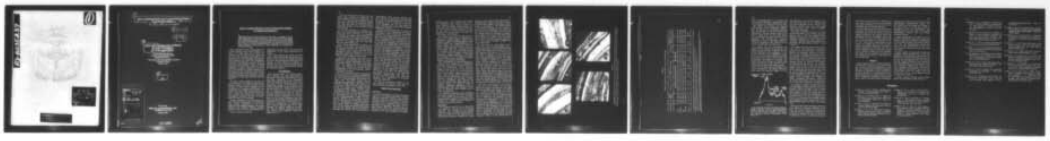
AD-A035 237 SCHOOL OF AVIATION MEDICINE RANDOLPH AFB TEX F/G 6/19
DENTAL CHANGES INDUCED IN RATS BY PROLONGED EXPOSURE TO ADVERSE--ETC(U)
DEC 58 N O HARRIS, R B MEFFERD, S R RESTIVO

UNCLASSIFIED 59-9 NL

1 OF 1
AD A035237



END
DATE FILMED
3-77

DENTAL CHANGES INDUCED IN RATS BY PROLONGED EXPOSURE TO ADVERSE ENVIRONMENTS

(11) Dec 58

(12) 10p.

(10) NORMAN O. HARRIS, Lieutenant Colonel, USAF (DC)*
ROY B. MEFFERD, JR., Ph.D.†
SALVATORE R. GESTIVO, M.S.†

*Research Dentistry Division
School of Aviation Medicine, USAF
Randolph AFB, Texas

†The Clayton Foundation Biochemical Institute
University of Texas
Austin, Texas

(14) 59-9

Air University
SCHOOL OF AVIATION MEDICINE, USAF
RANDOLPH AFB, TEXAS
December 1958

DENTAL CHANGES INDUCED IN RATS BY PROLONGED EXPOSURE TO ADVERSE ENVIRONMENTS

Histopathologic and chemical studies were made on teeth of four groups of rats following chronic exposure to varying environments of low (3° C.), neutral (24° C.), and high (35° C.) temperatures; or to reduced barometric pressure (380 mm. Hg) at these temperatures. Histologic changes were not seen in the ground control or cold groups. Altitude was associated with a disruption of the odontoblastic layer and with a loss of polarity. This effect was accentuated by superimposed cold. Heat counteracted some of these altitude effects, but caused ameloblastic changes. Chemically, the concentrations of calcium, phosphate, and magnesium were reduced significantly in the altitude- and heat-exposed groups.

Some individuals exposed to low barometric pressures undergo certain dental changes. In a study of 75 human teeth, most of which were extracted soon after descent from altitude and after the onset of painful aerodontalgia, Orban and Ritchey (1) found pathologic alterations in pulpal histology that varied from noninflammatory edema to vacuolization of areas in the horns of the pulp. After exposure of dogs to simulated altitude, there were definite changes in pulpal morphology, including hyperemia and hemorrhage (2). Exposure to altitude apparently did not change the rate of deposition of the organic matrix of the dentin in the rat incisor, but calcification of the dentin was disturbed, as shown by differences in the light and dark incremental lines (3).

The gross external appearance of teeth from rats exposed for 3 to 6 months to different environments (neutral, cold, heat, altitude, or combinations of these) varied strikingly with the environment; for example, heat-acclimated rats usually had heavy reddish-brown deposits over much of each tooth, while altitude rats did not. This occurred even though food intake (and consequently, masticatory activity) was approximately the same in the two groups.

Chemical alterations of the teeth should coincide with such apparent gross and histopathologic changes, especially in view of the significant and prolonged changes in electrolyte excretion rates observed following prolonged exposure of rats to adverse environments (4-7).

Received for publication on 22 July 1958.

Similarly, the urinary Ca/PO_4 ratio was elevated significantly in men during acclimatization to heat (8).

This report presents the results of experiments in which the histology and chemical composition of the incisors of variously acclimated rats were compared. Significant differences were observed in both respects. In addition, the mandible, the parotid and submaxillary glands, and the tongue were examined microscopically for evidence of pathology.

EXPERIMENTAL

Male Sprague-Dawley rats (initial weights, 400 to 450 gm.) were caged individually and maintained on Red Chain Dog Checkers or Purina Dog Chow, respectively, in two experiments. Uniform daily lighting and animal care schedules were used. The first experiment involved six environmental groups of rats exposed for 18 weeks to ground-level barometric pressure (750 mm. Hg) at low (3° C.), neutral (24° C.), or high (36° C.) temperatures, or to the same temperatures at reduced pressure (380 mm. Hg). The histologic condition of the teeth was evaluated, but their chemical composition was not determined. In the second experiment, there were four environmental groups exposed for 24 weeks respectively to low (3° C.), neutral (24° C.), and high (35° C.) temperatures, or to reduced barometric pressure (380 mm. Hg, 26° C.). Both histologic and chemical studies of the teeth were made. In addition, physiologic, biochemical, anatomic and histologic effects were examined (6, 7, 9).

The rats – both fasted (24 hours) and nonfasted – were weighed and decapitated. The intact mandible, a number of molars, and sections of the parotid and submaxillary glands, and tongue were taken for histologic examination. Since this short fast (24 hours) had no significant influence on the results, the data were combined.

In the first experiment the mandibles were fixed in 10 percent formalin, and were decalcified with formic acid. They were embedded in celloidin and stained with hematoxylin and eosin. Specimens from the neutral, cold, altitude, altitude plus cold, and altitude plus heat were examined (the samples from the heat group alone were inadvertently lost).

In the second experiment the mandibles were divided at the midline. The incisor from the left side was dissected, while the right half of the mandible was left intact. Both were sealed separately in tubes containing 10 ml. of 1M tetrasodium EDTA, pH 7.0 (10, 11). A year was allowed to effect complete decalcification.

Comparative descriptions were based on the appearance of the epithelial loop area, ameloblastic layer, dentin, odontoblastic layer, pulp, periodontal membrane, and osseous supporting tissue. The condyles and the various other tissues were examined for evidence either of histopathology or of environmentally specific compensatory alterations.

The EDTA decalcified incisors of experiment 2 were used for the chemical determinations. The EDTA solution was decanted and the matrix was washed repeatedly with acidified distilled water. The EDTA solution and the washings were combined and digested with hot concentrated HNO_3 - $HClO_4$ acid mixture (3:2) to destroy the EDTA and any organic material present. The samples were evaporated almost to dryness and then brought to 50 ml. volume with distilled water. Calcium was determined by EDTA titration (12, 13) and phosphate (as P) by colorimetry (14). The iron was determined as the bathophenanthraline complex (15) with iron-free reagents, but, since high concentrations of both calcium and phosphate interfere with the determination, it was necessary to remove them. Dowex 50 (200-400 mesh) resin in 1 x 10 cm. columns was used for this purpose. The resin was washed with 6N HCl until the washings were iron-free. This was followed by glass-distilled water until the washings were at pH 7. Ten ml. of each sample was run through the column for separation of the iron. This was followed by 10 ml. of N/10 HCl to elute most of the phosphate. The iron was eluted with 50 ml. of 4N HCl with a rate of flow of approximately 10 to 12 ml./hr.

Owing to the very large ratio of calcium to magnesium, accurate determination of the latter was difficult. Usual titration and precipitation methods were grossly inaccurate. Ten ml. aliquots from each sample were concentrated twofold by evaporation, and the magnesium in 60 μl. of this concentrated sample was separated from the calcium by a modified paper chromatographic method (16). The modification was necessitated by the fact that the large proportions of calcium decreased the distance of separation to a very thin line. Accordingly, the papers were sprayed directly with 8-hydroxyquinoline solution (0.5 percent in 60 percent methanol) and the magnesium spot was located under ultraviolet light when the moist strip was exposed to ammonia fumes. The fluorescent spots from two strips (total of 120 μl.) were removed, and the magnesium was eluted from the paper by five 2-ml. washings of 2N $HClO_4$. Ten ml. of concentrated nitric acid was added to destroy the 8-hydroxyquinoline, and the sample was evaporated to dryness in a sand bath. The soluble residue was brought up to 10 ml. and the magnesium determined colorimetrically with titan yellow (17). The protein content of the matrix was estimated by a modification of the Folin-Ciocalteau method (18).

The data were analyzed by analysis of variance technics, and suitable *t*-tests were run where indicated by significant F ratios (19).

RESULTS AND DISCUSSION

Experiment 1

The environmentally induced systemic changes observed in the different physiologic variables differed in magnitude, direction, time of onset, and duration (4-7, 9). In some cases (6) altitude exerted an effect that was additive to the thermal effects (e.g., nonfasting weight changes, phosphate and magnesium excretion, urine volume), while in others there was little apparent altitude effect per se (e.g., calcium and taurine excretion). The other variables had complex

responses; that is, the altitude effects varied with temperature. The Ca/Mg and the Ca/PO_4 ratios followed similar thermal patterns at ground level and altitude, except that they were on a lower plane at altitude. This resulted from the fact that the calcium excretion was not influenced by altitude while both magnesium and phosphate were elevated.

The teeth of the control and cold rats were normal in all respects (fig. 1). Regardless of temperature, altitude exposure induced histopathologic changes, including hyperemia of the pulpal tissues with a marked dilation of the vascular supply. The odontoblastic layer exhibited severe alterations in the distal third of the incisor. There was a tendency toward a loss of polarity, and also, in many areas, a tendency of the cells to assume a more cuboidal morphology. Increased vascularity of this layer in the distal third of the tooth was readily apparent. Rather severe invagination of dentin appeared to accompany these changes.

Cold superimposed on altitude appeared to accentuate the odontoblastic disorganization and loss of polarity, and to increase the vascularity and vacuolization. These alterations extended closer to the base of the tooth than they did in the altitude rats at neutral temperatures. Heat, however, apparently counteracted some of the altitude effects. Hyperemia was reduced and the odontoblastic layer was better organized, although the vascularity remained high. The ameloblastic layer, however, was severely disrupted. Many disorganized and discontinuous areas were evident in which the columnar morphology disappeared. Prior to decalcification, these teeth appeared to have areas of hypoplasia when examined under a dissecting microscope.

The periodontal membrane, bone morphology, and cellular components of the teeth as well as the various glands and the tongue of all environmental groups appeared to be normal except possibly for a slightly increased dilation of existing vessels in some groups.

Experiment 2

The conditions between the two experiments were different in that the rats of the second experiment were exposed for an additional month (24 weeks) at slightly different temperatures in the heat (35° C.) and altitude (26° C.). Though these differences seemingly were slight, changes so close to the critical regions exert a disproportionate influence. Histologic changes, in general, were similar to the respective counterparts in experiment 1. The thermal effects on electrolyte excretions (7) were similar to those of the first experiment (6), except that the Ca/PO_4 ratio was elevated in the cold, and the altitude effects on phosphate and magnesium excretion were reversed. The ratios (Ca/Mg and Ca/PO_4) were similar in the two experiments. The rats in this experiment appeared to be in better general physical condition than those of the former.

The food intake of cold-acclimated rats is about twice that of the other groups, and accordingly they excrete more of each of the electrolytes. The urinary ratios (Ca/Mg and Ca/PO_4) were elevated in this group (i.e., calcium excretion failed to increase as much as the other two). This is indicative of a possible disproportion during nonfasting periods, but which, even assuming it existed for a considerable period, caused no change in the chemical composition of the teeth (table I). There was a slight but consistent increase in all three ions (Ca, Mg, and PO_4) in the teeth of the cold group – which might indicate that relative to the organic matrix, the mineral content of the bone and/or dentin increased. In addition to the elevated metabolic rate, a secondary effect of the cold was a mechanical one, possibly influencing teeth and salivary glands. This was due to the greatly increased gnawing and chewing that accompanied the marked increase in food intake.

Food intake was depressed in the heat (two-thirds that of the control), and accordingly phosphate excretion was depressed, but magnesium was unchanged and calcium was elevated. As a result the ratios (Ca/Mg and Ca/PO_4) were elevated in the heat. Again this is indicative of an imbalance which, if prolonged, might lead to changes in the composition of teeth. Actually, the concentrations of all three ions (especially of magnesium) in the teeth were reduced. Thermal differences did not influence significantly the molar Ca/PO_4 ratio, since the changes in each electrolyte were proportional. Thus, there was more (cold) or less (heat) inorganic constituents per unit weight of tooth, but its composition was constant. The main thermal difference was in the greatly depressed magnesium content in the heat-exposed rats.

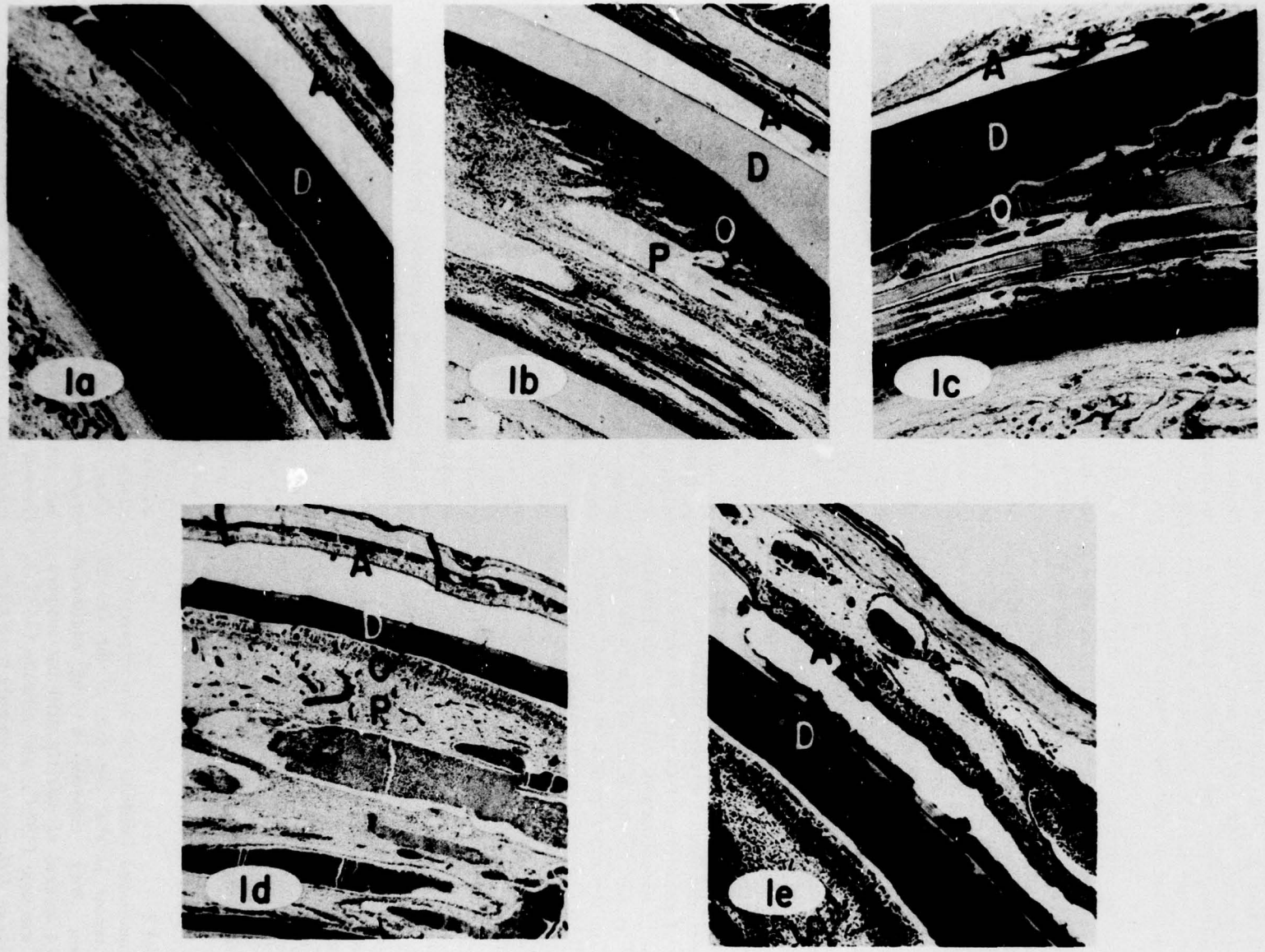


FIGURE 1

Longitudinal sections of the right incisor of rats acclimated to adverse environments (41× except for altitude + heat which is 64×; hematoxylin and eosin). 1a. *Cold.* 1b. *Neutral.* 1c. *Altitude.* 1d. *Cold + altitude.* 1e. *Heat + altitude.* **A**- *Ameloblastic layer.* **D** - *Dentin.* **O** - *Odontoblastic layer.* **P** - *Pulp.*

TABLE I

Chemical composition of the right incisors of acclimated rats

Environment	n	Body weight (gm.)	Protein	Calcium	Phosphate (as P)	Magnesium	Iron	Molar ratios		
								Ca/Mg	Mg/PO_4	Ca/PO_4
			(mg./100 mg. demineralized tooth)				(μg./100 mg.)			
Cold	39	413(33)*	15.8(2.3)	57.6(5.8)	29.0(2.8)	3.18(.13)	84.4(25.0)	10.9(.5)	.141(.010)	1.55(.07)
Neutral	18	464(27)	16.2(1.7)	57.2(6.5)	28.8(3.5)	3.15(.13)	87.6(20.3)	10.9(.5)	.141(.011)	1.55(.06)
Hot	35	426(28)*	16.4(2.3)	53.9(5.7)*	26.9(2.9)*	1.83(.08)*	89.2(30.6)	17.4(.8)*	.088(.009)*	1.57(.08)
Altitude†	18	388(24)*	15.4(3.2)	49.0(5.6)*	25.1(2.4)*	2.36(.10)*	76.7(22.1)	12.4(.6)*	.121(.011)*	1.52(.06)*
F ratio		19.0*	.90	14.0*	8.67*	4.38*	1.01	4.78*	7.61*	3.45*

The mean values are presented with the standard deviations in parentheses. The molar ratios were obtained with PO_4 expressed as P. The ratios are the averages of the individual ratios, so the values are slightly different from those obtained from the means themselves.

*The mean is significantly different from the control (neutral), or the F ratio was significant, $P \leq .05$.

†When expressed as mg. of ion/mg. protein, the altitude group increased by about 5 percent relative to the neutral group, while the heat group decreased by about 1 percent and the cold group increased by about 1 percent. The statistical relationship remained significant, however.

This is interesting when it is correlated with the fact that ameloblastic degeneration occurs in the presence of a magnesium deficiency (20).

The altitude rats, however, had depressed concentrations of all constituents measured. This was partly due to the depressed food intake, but the ratio of Ca/PO_4 was depressed significantly. Thus the inorganic phase of the bone or dentin itself may have been altered (i.e., the reduction in calcium exceeded that of phosphate). The excretion rates also were depressed, but even so, apparently insufficient calcium and magnesium were retained to maintain tooth metabolism at the proper level.

The protein and iron concentrations had almost identical patterns (linear increase with temperature, and decrease at altitude) but none of the differences were significant. It was believed that some of the reddish-brown deposits on the teeth of heat-acclimated rats might have resulted from excess iron deposits. The concentration was highest in this group. This deposit did not occur solely as a result of the reduced masticatory activity (reduced food intake), since the altitude rats had no deposits.

When the data were expressed relative to the protein concentration, the thermal differences noted above were magnified slightly (i.e., the concentrations in the cold were higher and in the heat lower), but the low altitude values were raised materially (about 5 percent). The depressed altitude values were still significantly below the control, however, regardless of the form of expression.

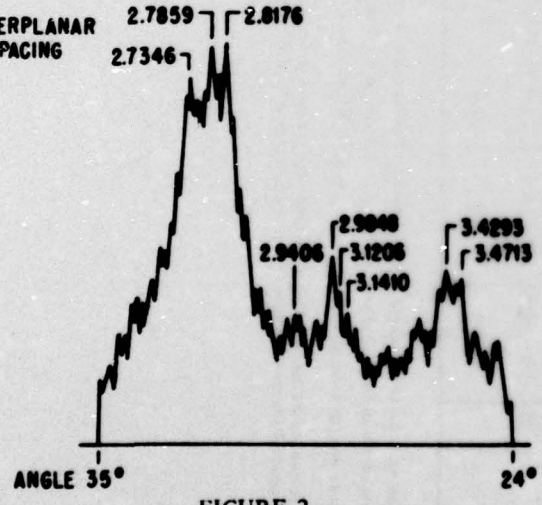


FIGURE 2

X-ray diffraction spectrum of rat incisors (hydroxyapatite). Angle from 24 to 35°. Interplanar distances (peaks) in angstroms. 200 mesh dentin + enamel from 15 pooled right incisors. Spectrum at other angles lacks distinctive features. Fluoroapatite is not discernible.

The copper concentration proved to be below the detection limits of our method, even when material from 5 teeth were combined. X-ray diffraction patterns of the teeth failed to reveal significant differences in basic molecular or crystalline structure. Only hydroxyapatite was discernible. One representative pattern is shown in figure 2.

Few studies have been reported where efforts were made to relate chemical and histologic changes above the cellular level. Climatic influences are notoriously difficult to evaluate, since so many extraneous uncontrollable factors are present (e.g., varying food intake, exercise, and humidity). Even in controlled environments such as were used in these experiments, confounding factors are involved. For example, the heat-exposed rats ate less, and, therefore, chewed less, so that chemical and histologic changes could be due to any one of several factors. Among these are the smaller food intake, per se, the decreased mechanical attrition, or the altered chemical erosion caused by a change in the composition or pH of the saliva or by a change in the microbial flora. Furthermore, various nutritional deficiencies, induced by the reduced food intake or by the environment per se, could have contributed to the differences.

Far too little is known at present about amino acid metabolism to permit speculation about the possible relation of changes in the excretion patterns of one or the other of these to histologic changes of teeth. The general picture in the altitude rats (where the histopathology was greatest) however, was that of nitrogen depletion (4, 21) as evidenced by the slightly reduced matrix polypeptide level. Changes such as this understandably could lead to the tissue damage seen.

Changes in the over-all electrolyte metabolism were related to structural alterations in the teeth. The exterior evidence of decalcification in the heat rats was verified by the reduced concentration of calcium, but since the phosphate concentration was not reduced proportionately,

the Ca/PO_4 ratio decreased. It is unlikely that calcium was replaced to any extent by magnesium, since the Ca/Mg ratio was elevated, and the Mg/PO_4 ratio was reduced markedly.

The heat-exposed rats also had reduced concentrations of all three ions, but in contrast to the altitude rats, and in line with their increased calcium retention (7), the calcium content was influenced the least. Accordingly, the Ca/Mg and Ca/PO_4 ratios were elevated, although the latter was just below the significance level ($P > .05$). The decreased concentration of magnesium was striking, but the cause of these changes is unknown. Magnesium is required to prevent hyperthermia in the heat (22), and its reduced excretion in this group (4-8) is suggestive of retention. Perhaps, with the depressed food intake and the increased metabolic need, the magnesium was utilized at the partial expense of the teeth.

SUMMARY

Marked changes occur in calcium, phosphate, and magnesium excretions during chronic exposure of rats to adverse environments. Accordingly, the histology and the chemical composition of teeth were examined from rats acclimated to cold, heat, altitude, or to combinations of these. Chronic exposure to the conditions (3°, 24°, and 36° C. at barometric pressures of either 750 or 380 mm. Hg) for 18 weeks resulted in negligible changes in the cold, but at altitude there were pathognomonic histopathologic changes that were specific for the mesenchymal elements of the teeth. These were made more severe by superimposed cold, but superimposed heat counteracted some of the effects. In this group (heat-altitude), however, there were marked ectodermal changes.

Longer term exposure (24 weeks at 3°, 24°, and 35° C. and 380 mm. Hg at 26° C.) apparently resulted in either more complete acclimatization, or in the death of more of the poorly acclimatized rats. The histopathologic changes had many of the same characteristics as were noted in the previous experiment, but they were much less severe.

Chemical studies on these teeth revealed significantly reduced concentrations of calcium, phosphate, and magnesium in the altitude- and heat-exposed rats. The cold-acclimated rats, as was the case histologically also, did not differ from the controls. Protein and iron content did not differ significantly among the groups. There were significant changes in the ratios of calcium, magnesium, and phosphate. Possibly the basic inorganic composition of the teeth of altitude-acclimated rats was changed since the Ca/PO_4 ratio was low. X-ray diffraction failed to confirm this, however.

Possible relations between these changes and the excretion patterns were discussed.

REFERENCES

1. Orban, B., and B. T. Ritchey. Toothache under conditions simulating high altitude flight. J. Am. Dent. A. 32:145-180 (1945).

2. Orban, B., B. T. Ritchey, and A. A. Zander. Experimental study of pulp changes produced in the decompression chamber. J. Dent. Res. 25:299-309 (1946).

3. Gersh, L., and J. F. Restarski. The effects of simulated altitudes upon the incisor of the rat. Anat. Rec. 90:191-195 (1944).

4. Mefferd, R. B., Jr., H. B. Hale, and H. H. Martens. Nitrogen and electrolyte excretion of rats chronically exposed to adverse environments. Am. J. Physiol. 192:209-218 (1958).

5. Mefferd, R. B., Jr., and H. B. Hale. Fffects of altitude, cold, and heat on metabolic interrelationships in rats. Am. J. Physiol. 193: 443-448 (1958).

6. Hale, H. B., and R. B. Mefferd, Jr. A factorial study of environmentally induced metabolic changes in rats. School of Aviation Medicine, USAF, Report No. 58-54, Feb. 1958.

7. Mefferd, R. B., Jr., and H. B. Hale. Studies on cross-adaptation: Effects of abrupt temperature changes on excretion characteristics of rats acclimated to cold, neutral, or hot environments. School of Aviation Medicine, USAF, Report No. 58-106. (In press)

8. Mefferd, R. B., Jr., E. LaBrosse, A. Gawienowski, and R. J. Williams. Influence of chlorpromazine on certain biochemical variables of chronic male schizophrenics. J. Nerv. & Ment. Dis. (In press)

9. Criscuolo, D., H. B. Hale, and R. B. Mefferd, Jr. Indices of oxygen transport, utilization, and storage in rats acclimated to altitude at different temperatures. School of Aviation Medicine, USAF, Report No. 58-94, July 1958.

10. Hahn, F. L., and F. Reygadas. Demineralization of hard tissues. Science 114:462-463 (1951).

11. Sreebny, L. M., and G. Nikiforuk. Demineralization of hard tissues by organic chelating agents. Science 113:560 (1951).

12. Schwarzenbach, G., W. Biederman, and F. Baugerter. Komplexone, VI. Neue einfache Titrier methoden zur Bestimmung der Warserhirte. Helvet. chim. acta 29:811-818 (1946).

13. Betz, J. D., and C. A. Noel. Total hardness determination by direct colorimetric titration. J. Am. Water Works Assoc. 42:49-56 (1950).

14. Fiske, C. H., and Y. Subbarow. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400 (1925).

15. Smith, C. F., W. H. McCurdy, and H. Diehl. The colorimetric determination of iron in raw and treated municipal water supplies by use of 4:7-diphenyl-1:10-phenanthraline. Analyst 77: 418-422 (1952).

16. Pollard, F. H., J. F. W. McOmie, and J. V. Martin. The quantitative analysis of the alkaline-earth metals by paper chromatography. Analyst 81: 353-358 (1956).

17. Kunkel, H. O., P. B. Pearson, and B. S. Schweigert. The photometric determination of magnesium in body fluids. J. Lab. & Clin. Med. 32:1027-1033 (1947).

18. Oyama, V. I., and H. Eagle. Measurement of cell growth in tissue culture with a phenolreagent (Folin-Ciocalteau). Proc. Soc. Exper. Biol. & Med. 91:305-307 (1956).

19. Lindquist, E. F. Design and analysis of experiments. Boston: Houghton-Mifflin Co., 1953.

20. Kleen, H., E. R. Orent, and E. V. McCollum. The effects of magnesium deficiency on the teeth and their supporting structures in rats. Am. J. Physiol. 112:256-262 (1935).

21. Tappan, D. V., B. Reynafarje, V. R. Potter, and A. Hurtado. Alterations in enzymes and metabolites resulting from adaptation to low oxygen tensions. Am. J. Physiol. 190:93-98 (1957).

22. Hall, V. E., R. Grant, and W. J. Whalen. The influence of magnesium and pyrogens on temperature regulation. A. F. Technical Report No. 6682. Wright Air Development Center, Air Research and Development Command, USAF, Wright-Patterson AFB, Ohio, 1951.